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TECHNICAL MANUSCRIPT 28

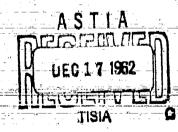
THE SEROLOGICAL ASSAY

OF CULTURE FILTRATES FOR

STAPHYLOCOCCUS ENTEROTOXIN

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UNITED STATES ARMY BIOLOGICAL LABORATORIES FORT DETRICK

U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY U.S. ARMY BIOLOGICAL LABORATORIES Fort Detrick, Maryland

The work reported here was performed under Project 4B11-02-066, Special BW Operations, Research on Staphylococcus Enterotoxin. The expenditure order was 2057.

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Project 4B11-02-066

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ANIMAL RESEARCH

In conducting the research reported herein, the investigators adhered to "Principles of Leberatory Animal Care" as established by the National Society of Medical Rusearch.

The agar diffusion technique of Oudir has been used by Surgalla et al³ to determine the relative concentrations of antigens in preparations of staphylococcal enterotoxin and to study its antigenic properties. Bergdoll et al^{4,5} using the same technique, identified a precipitate band with the emesis-producing component of partially purified toxin and used this procedure as a guide in the purification of enterotoxin.

Since highly purified type B enterotoxin produced by the S6 strain of Staphylococcus aureus was available, estimations of enterotoxin by the gel diffusion and quantitative precipitin test were compared.

Purified enterotoxin prepared by Dr. E. J. Schantz, Fort Detrick, by a modification of the procedure of Bergdoll was dissolved in the desired concentrations in 0.02 M phosphate-buffered saline, pH 7.4, containing 1: 10,000 merthiclate. Antitoxin was obtained by the extensive immunization of rabbits with this preparation, emulsified in Freund's complete adjuvant. The initial dose of 37.6 micrograms was increased by fivefold amounts until a maximum dose of 3.0 milligrams was administered. Each rabbit received a total of 19.5 milligrams of toxin over a six-month period. All injections were given by the subcutaneous route; the time interval and dose were dependent upon the reaction of the individual rabbit. After each injection the animals responded with an increase in body temperature and a loss of weight. The pooled sera contained 0.68 milligram of antibody N per milliliter as determined by the quantitative precipitin test of Heidelberger and Kendall.

Standard curves for both the gel diffusion test and the pracipitin; test were prepared with the purified toxin and antitoxin. Agar diffusion tests were incubated at 30°C in a glass-walled water bath. The movement of the pracipitate band was measured at about 24-hour intervals using a cathetometer A single band of pracipitate was formed in the interaction of these two reagents. Quantitative pracipitin tests were incubated at 37°C for four hours plus 4°C for four days. Washed pracipitates were analyzed for total N by the micro-kjeldahl technique. Tests of the supermatant serum obtained after centrifugation and removal of the pracipitate never showed the simultaneous presence of both antigen and antibody.

The comparative tests were performed on a number of filtrates of §, sursus cultures grown in various media at 37°C for 18 to 24 hours. The cultures were centrifuged, and the clarified fluid was sterilized by filtration through a UF sintered glass fi)ter. Undiluted filtrate or filtrate diluted 1:5 with the buffered saline was tasted by the Oudin test. In the quantitative precipitin tests, 0.5-milliliter amounts of undiluted or diluted filtrate (1:5) were mixed with 0.5 milliliter of antitoxin.

Representative results are presented in Table I. Analysis of the data by the "t" test showed no significant difference between the results obtained with the two tests at the 95 per cent level. These tests indicated that excellent correlation existed between the results obtained by the gel diffusion and the quantitative precipitin tests.

TABLE 1. COMPARISON OF STAPHYLOCOCCUS ENTEROTOXIN DETERMINATIONS BY THE GEL DIFFUSION AND THE QUANTITATIVE PRECIPITIN TESTS

SAMPLE	Dilution	μα enterotoxin per ml	
		Gel Diffusion	Precipitin
В		143.0	153.8
В	1:5	150.0	
A		57.5	90.8
A	1:5	48.0	
F		180.0	169.6
F	1:5	173.5	
C		19.0	24,8
1		24.5	27.6
2		48.0	46.8
3		37.0	33.6
5		44.0	41.5
7		31.5	35.6
OA		15.9	22.4

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